

RUSSELL PRESSEY

Red River Valley Potato Processing Laboratory, East Grand Forks, Minnesota 56721

Purification and Properties of Phosphoglucomutase from Potato Tubers

SUMMARY—The level of phosphoglucomutase in potato tubers has been found to be about 400 times higher than previously reported. This enzyme has been purified 40-fold over the dialyzed crude extract by a procedure consisting of fractionation with ethanol and acetone, fractionation with ammonium sulfate, and filtration through Sephadex G-100. The potato enzyme is similar to phosphoglucomutases obtained from other sources, but most closely resembles the rabbit muscle enzyme. It requires Mg^{2+} and cysteine for activity, is activated by preincubation with Mg^{2+} and cysteine, possesses a molecular weight of 63,000, exhibits optimal activity at pH 7.6, and is inhibited by anions.

INTRODUCTION

THE ENZYME PHOSPHOGLUCOMUTASE (PGM), which catalyzes the reversible transfer of a phosphate group between the 1- and 6-positions of glucose, has been identified in a variety of organisms. Because of its general occurrence in biological systems, this enzyme was recently selected for a comparative study of an enzyme from a diversity of organisms (Joshi and Handler, 1964; Joshi *et al.*, 1965). It was found that the enzymes isolated from rabbit muscle, flounder, yeast, and *E. coli* are similar in many respects.

Although PGM has been crystallized and characterized from rabbit muscle (Najjar, 1948) and *E. coli* (Joshi and Handler, 1964), detailed studies on PGM in higher plants are lacking. Some of the properties of PGM in crude extracts of *Phaseolus radiatus* (Ramasarma *et al.*, 1954) and jack-bean (Cardini, 1951) have been described. The only attempt to purify the plant enzyme was made by Boser (1957) who reported a 900-fold enrichment of potato tuber PGM. Despite the great purification achieved, the purified enzyme possessed feeble activity in comparison to crystalline rabbit muscle PGM.

The conversion of starch to glucose and fructose in

potatoes during cold storage proceeds through a sucrose intermediate (Pressey and Shaw, 1966). Assuming that phosphorylase action on starch yielding glucose-1-phosphate is the first step in this transformation, then participation of PGM is required for fructose formation prior to sucrose synthesis. Preliminary experiments on PGM in potato tubers indicated a much higher level of this enzyme than previously reported (Boser, 1957). For this reason, it was decided to re-examine the purification and properties of potato PGM. It was found the enzyme can be purified to a specific activity comparable to preparation from other sources. Properties of the potato enzyme are presented and compared to those of the PGM's that have been characterized.

MATERIALS AND METHODS

GLUCOSE-1-P, cytochrome c, ovalbumin, and serum albumin were purchased from Nutritional Biochemicals Corp. The glucose-1-P contained a sufficient quantity of glucose-1, 6-di-P for optimal PGM activity. Histidine and imidazole were obtained from Eastman Organic Chemicals and L-cysteine was obtained from Sigma Chemical Co.

Assay of PGM activity was performed by measuring the conversion of glucose-1-P to glucose-6-P. Unless otherwise indicated, the standard reaction was carried out at 30°C and pH 7.6 in a mixture that contained the following components expressed as μ moles: L-cysteine, 15; magnesium acetate, 10; glucose-1-P, 10; in a final volume of 3.1 ml. After preincubation at 30°C for 10 min., 0.1 ml of enzyme appropriately diluted with a solution containing 0.01M Mg^{2+} and 0.01M cysteine, pH 7.6 was added. The reaction was terminated after 5 min by adding 1 volume of 0.2M sodium acetate, pH 4.7, and heating for 2 min in a boiling water bath. Glucose-6-P was determined in a

suitable aliquot by measuring the acid-stable and acid-labile phosphate (Fiske and Subbarow, 1925) or by measuring the reducing sugars (Nelson, 1944) using glucose-6-P as a standard. A heated enzyme control was run with every assay. Enzyme activity was linear with protein concentration and with time (Fig. 1).

One unit of PGM activity is defined as that amount of enzyme required for the formation of 1 μ mole of glucose-6-P in 1 min under standard conditions.

Protein concentration was measured by the biuret method (Layne, 1957) in relatively crude extracts and by determining the ratio of absorbancies at 280 and 260 $m\mu$ (Warburg and Christian, 1942) in more purified preparations. Crystalline serum albumin was used as a standard in both methods. Specific activity is expressed in units per mg protein.

Purification of PGM

Unless otherwise indicated, all operations were carried out at 2°C and centrifugations were performed at 10,000 xg for 20 min.

Step 1. Crude extract. Mature potato (*Solanum tuberosum* L. var. Kennebec) tubers were harvested in September, 1965, and stored in the dark at 4°C for about 4 months. Washed, peeled tubers (750 g) were sliced and passed through a juicerator (Acme Juicer Manufacturing Co., Lemoyne, Pa.). Six ml of 0.8M sodium sulfite, pH 6.0, were added to the juice to prevent enzymatic darkening. The pH of the extract was adjusted to 7.6 with Tris and the juice was clarified by centrifugation. The solution was then dialyzed against 7 L of 0.01M magnesium acetate containing 0.01M L-cysteine, pH 7.6. After 6 hr, the dialyzing solution was changed and dialysis was continued overnight.

Step 2. Alcohol and acetone. The dialyzed extract (250 ml) was cooled to 0°C in a cold bath and 125 ml of precooled 95% ethanol were added slowly, allowing the temperature to drop to -10°C. The precipitate was removed by centrifugation at 4000 xg for 20 min at -10°C. This precipitate contained over 90% of the protein, but only about 40% of the PGM. To the supernatant were added 750 ml of precooled acetone and the sample was

cooled to -15°C. After 2 hr, the precipitate was collected by decantation and centrifugation at -15°C and 2000 xg for 5 min. The precipitate was dissolved in 100 ml of cold 0.01M magnesium acetate and 0.01M L-cysteine, pH 7.6.

Step 3. Ammonium sulfate. The acetone fraction was made to 55% saturation of ammonium sulfate by adding saturated solution at pH 7.6. After 15 min, the resulting suspension was centrifuged and the precipitate was discarded. Saturated ammonium sulfate was added to the supernatant to 75% saturation. The precipitate was collected by centrifugation and dissolved in 15 ml of 0.01M magnesium acetate and 0.01M L-cysteine, pH 7.6.

Step 4. Sephadex G-100. The ammonium sulfate fraction was applied to a 2.5 \times 90 cm column of Sephadex G-100 washed with 0.01M magnesium acetate containing 0.01M L-cysteine, pH 7.6. Elution was accomplished with the wash solution, and 11-ml fractions were collected with an automatic fraction collector. The protein elution pattern consisted of one major peak followed by a minor peak. The enzyme was eluted between the two protein peaks but with considerable overlap of the enzyme and the larger protein peaks. The two fractions of highest specific activity were combined and stored at -20°C.

RESULTS

Activation of PGM in crude extracts

In preliminary experiments on potato PGM, it was observed that the enzyme activity was influenced by Mg^{2+} and cysteine in the solutions used for dialyzing crude extracts. PGM in extracts dialyzed against 0.01M magnesium acetate containing 0.01M L-cysteine, pH 7.6, possessed a specific activity of 2.4 compared to about 0.8 for the enzyme in extracts dialyzed against water or either Mg^{2+} or cysteine alone. Both Mg^{2+} and cysteine were required for activation, but cysteine was equally effective over the concentration range of 0.005 to 0.05M in the presence of 0.01M Mg^{2+} .

Other compounds that produced activation of PGM in the presence of 0.01M Mg^{2+} were 0.01M histidine and 0.005M imidazole. The order of effectiveness was cysteine, histidine, and imidazole, with the imidazole effect equal to about half of the cysteine effect. Histidine and imidazole were not effective in the absence of Mg^{2+} . Moreover, 0.05M and higher concentrations of imidazole alone decreased PGM activity. EDTA over a wide range of concentration in the presence and absence of Mg^{2+} did not have an effect on PGM activity. This observation suggests an activation mechanism of Mg^{2+} and cysteine other than heavy metal ion binding.

The activated enzyme was deactivated on dialysis against water or 0.01M Mg^{2+} . The deactivated enzyme could be reactivated by preincubation with Mg^{2+} and cysteine, imidazole, or histidine. However, the activity of reactivated enzyme was generally lower than that of the original activated enzyme. Nevertheless, it is evident that the activation process is reversible and that the continuous presence of optimal concentrations of Mg^{2+} and cysteine is required for a maximally activated state.

It is clear that the potato enzyme is similar to the rabbit muscle PGM in that both are activated by preincubation with Mg^{2+} in the presence of cysteine, histidine or imida-

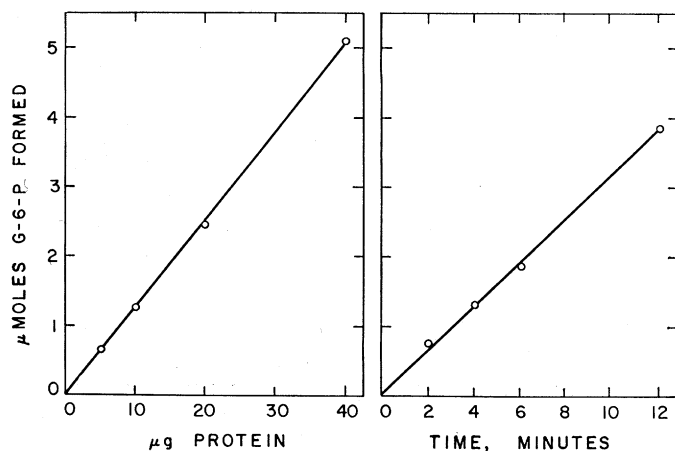


Fig. 1. Linearity of PGM activity with protein concentration and time. The conditions of the experiment were standard and the enzyme employed was an ammonium sulfate fraction with a specific activity of 25.

zole. (Harshman *et al.*, 1965). As in the case of the rabbit enzyme, the activation of potato PGM involves a process different from the catalytic stimulation of enzyme activity. Thus, Mg^{2+} and cysteine are both required for catalytic activity, but stimulation by these reagents in the reaction solution is never as high as the activation of the enzyme on preincubation.

Purification of the enzyme

Because of the increased stability of the activated enzyme, this form was chosen for the purification of potato PGM. A summary of a typical purification is presented in Table 1. A 40-fold increase in specific activity with 18%

Table 1. Purification of the enzyme.

Step	Volume (ml)	Total Protein (mg)	Total Units	Specific Activity (units/mg)	Recovery Enzyme (%)
1. Crude extract	250	2,550	6,100	2.4
2. Alcohol and acetone	100	225	3,700	17	61
3. Ammonium sulfate	15	120	2,900	24	48
4. Sephadex G-100	22	11	1,100	100	18

recovery was obtained. The partially purified enzyme was free of glucose-1-phosphatase and glucose-6-phosphatase. However, the Sephadex G-100 fraction undoubtedly contains other proteins because the enzyme peak occurred on the shoulder of a large protein peak. Further purification was possible by chromatography on DEAE-cellulose, but poor recovery of the activity did not warrant the use of this step.

Molecular weight

The molecular weight of the enzyme was estimated by gel filtration through Sephadex G-100 (Whitaker, 1963). Proteins of known molecular weight used to calibrate the column were serum albumin (70,000), ovalbumin (45,000), and cytochrome c (13,000). When the elution volumes of the proteins were plotted against the logarithms of their molecular weights, a straight line was obtained (Fig. 2). Gel filtration of purified PGM, under identical conditions, gave an elution volume of 198 ml which corresponds to a molecular weight of 63,000.

Effect of pH

The pH optimum of potato PGM was found to be about 7.6 in 0.07M Tris-acetate buffers (Fig. 3). The activated and unactivated enzymes exhibited identical optima.

Kinetics

Pure glucose-1-P and glucose-1,6-di-P were not available to determine the effect of substrate concentration on enzyme activity. However, a number of observations indicate that glucose-1,6-di-P is required for maximum activity of the potato enzyme, in accord with the accepted mechanism of PGM action (Najjar and Pullman, 1954). Very low activity was obtained with some samples of commercial glucose-1-P and this is attributed to low levels of

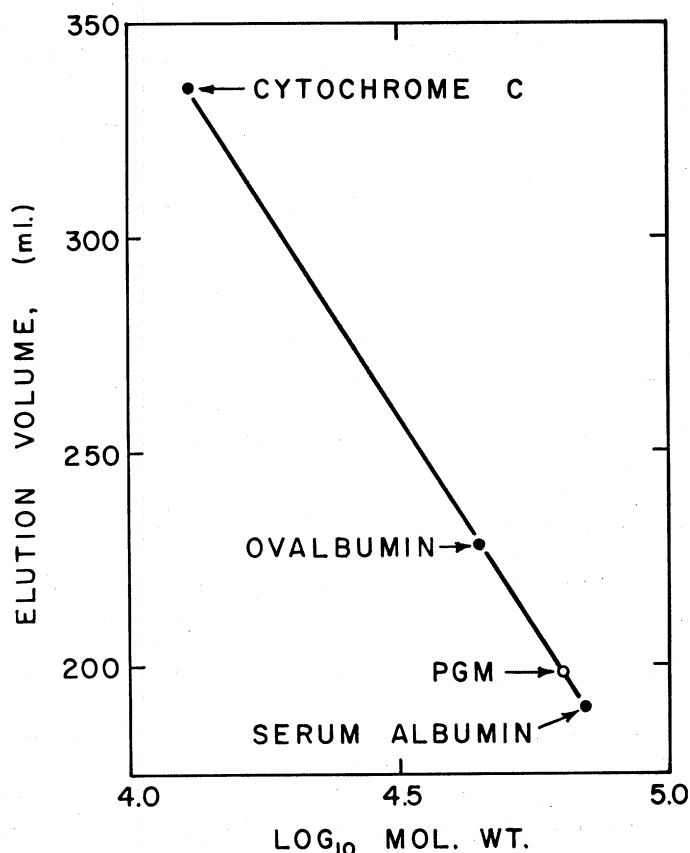


Fig. 2. Determination of the molecular weight of potato PGM by gel filtration on Sephadex G-100. The column (2.5 × 99 cm) was washed with 0.01M Mg^{2+} containing 0.01M cysteine, pH 7.6. Each of the standard proteins (25 mg) was applied in a volume of 5 ml and their elution was followed by measuring the absorption at 280 m μ . Five ml of purified PGM was applied to the column and elution was followed by the standard enzyme assay. Elution was accomplished with 0.01M Mg^{2+} and 0.01M cysteine, pH 7.6.

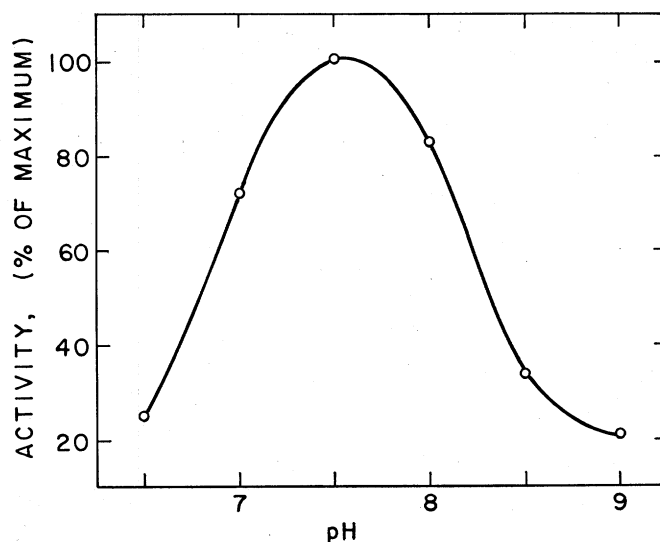


Fig. 3. Effect of pH on the activity of potato PGM. The pH was varied by the inclusion of 0.07M Tris-acetate in the standard reaction mixture.

the diphosphate. Furthermore, when the concentration of a mixture of glucose-1-P and glucose-1,6-di-P was varied over a wide range, reciprocal plots with upward curvature

were obtained. These curves were similar to those described by Dixon and Webb (1964) for an enzymatic reaction in which the substrate is also an activator. Curves that diverge upward are also predicted from data presented on rabbit muscle PGM by Ray and Roscelli (1964) if both glucose-1-P and glucose-1,6-di-P are varied simultaneously.

Requirements for activity

As described earlier, preincubation of potato PGM with Mg^{2+} and cysteine, histidine, or imidazole results in activation of the enzyme. This induced activation does not eliminate the requirement of Mg^{2+} and cysteine, histidine, or imidazole in the reaction solution for enzyme activity. Enzyme deactivated by dialysis against water exhibited maximal activity in the presence of $4 \times 10^{-3}M$ cysteine (Fig. 4) and $2 \times 10^{-3}M$ Mg^{2+} (Fig. 5). Enzyme activity was zero in the system lacking cysteine and very low in the system lacking Mg^{2+} . Mg^{2+} at concentrations higher than $0.05M$ inhibited PGM activity. Cysteine could be substituted by $0.04M$ imidazole or $0.01M$ histidine, although the response to imidazole was lower than for histidine and cysteine.

Inhibitors

Potato PGM is similar to enzymes from other sources in sensitivity to anions in the assay medium (Table 2). Borate is an especially effective inhibitor.

DISCUSSION

THE RESULTS PRESENTED HERE are at variance with the findings of Boser (1957) regarding the level of PGM in potato tubers. Whereas Boser reported a specific activity of 0.0021 (μ moles glucose-6-P/mg/min) for PGM in crude extracts, we have found that the specific activity of crude, unactivated PGM is 0.8 or about 400 times higher. The specific activity of our partially-purified PGM is 50

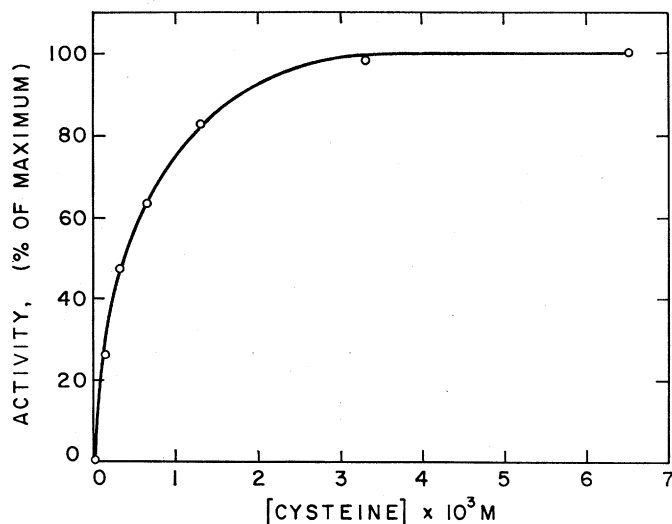


Fig. 4. Effect of cysteine concentration on the activity of potato PGM. The enzyme was a Sephadex G-100 fraction and was deactivated by dialysis against water. Assay conditions were standard except for the variation of cysteine concentration.

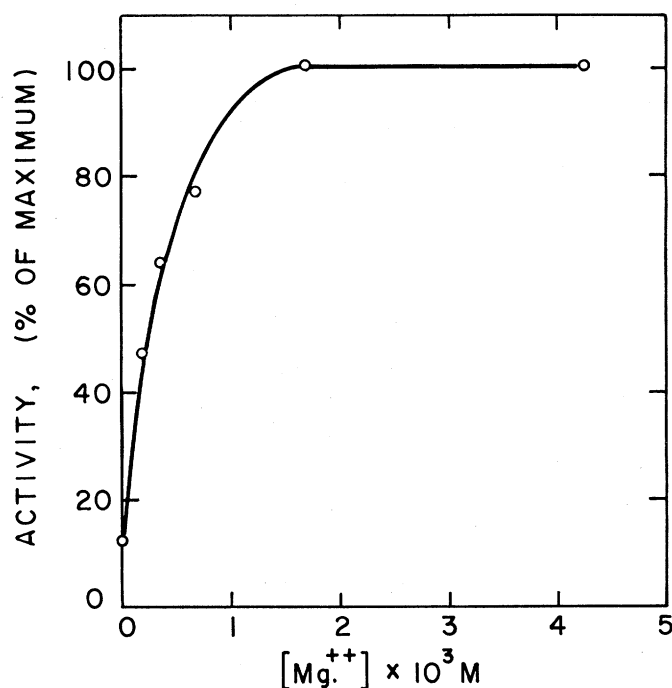


Fig. 5. Effect of Mg^{2+} concentration on the activity of potato PGM. The enzyme was a Sephadex G-100 fraction and was deactivated by dialysis against water. The assay conditions were standard except for the variation of Mg^{2+} concentration.

Table 2. Effect of anions on PGM activity.

Anion	Concentration	Percent of Control Activity
Acetate	0.1M	100
Chloride	0.1M	87
Nitrate	0.1M	63
Sulfate	0.05M	56
Sulfite	0.05M	50
Phosphate	0.05M	47
Borate	0.005M	36

The conditions of the experiment were those of the standard assay procedure. Anions were added to the specified levels as solutions of the sodium salts at pH 7.6.

times higher than Boser's purified enzyme. It is difficult to explain why Boser found only a negligible level of PGM in potato tubers. His assays were conducted at concentrations of Mg^{2+} and cysteine sufficiently close to optimal. It is possible that the variety of potato tubers selected by Boser for PGM purification possesses low activity. The most likely reason for the low activity is the use of glucose-1-P preparations containing very low levels of glucose-1,6-di-P.

The specific activity of the purified potato PGM is 2.5 times higher than that reported for crystalline *E. coli* enzyme (Joshi and Handler, 1964). However, this activity is considerably lower than that reported for activated rabbit muscle enzyme (Robinson, *et al.*, 1965). Many of the properties of potato PGM are similar to those of enzymes isolated from rabbit muscle, *E. coli*, yeast, and flounder. The potato enzyme most closely resembles rabbit muscle PGM. Features that are strikingly similar are: molecular weight; activation by Mg^{2+} in combination with cysteine,

imidazole, or histidine; requirement of Mg^{2+} and cysteine, imidazole, or histidine for activity; pH optimum; and inhibition by anions. One of the few differences between the two enzymes is the relative effectiveness of cysteine and imidazole as activators. Imidazole is more effective for the rabbit muscle PGM, but cysteine is more effective for the potato enzyme.

The differences between the potato and *E. coli* enzymes are more numerous and significant. The pH optimum for the bacterial enzyme is at 9.0 compared to 7.6 for potato PGM. The *E. coli* enzyme is stimulated by cysteine, but not by histidine or imidazole. Perhaps the most important difference between the two enzymes is the insensitivity of the bacterial PGM to preincubation with Mg^{2+} and cysteine, histidine, or imidazole. However, the two enzymes possess similar molecular weights and both are inhibited by anions.

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